RESISTANCE OF RICE GENOTYPES TO THE BLAST FUNGUS AND THE ASSOCIATED BIOCHEMICAL CHANGES

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Abstract

R ice blast is the most important disease in Egypt and worldwide. The rice blast is caused by *Pyricularia grisea* this fungus can produce physiological races. Development of varietal resistance is the most effective way to control rice blast. This study was focused on identification of 20 isolates and evaluation of 21 rice genotypes for blast disease. Also, determining the biochemical changes after inoculation with the blast pathogen. The isolates were identified as five groups, IC group race the common followed by ID and only one race for each IA, IB and IF. Evaluation for genotypes showed that the first and second groups were resistant to blast infection. However, the third group showed 70 to 75% resistant. Biochemical changes included determination of antioxidant enzymes (Peroxidase (POX) and Ascorbate peroxidase (APX), defense-related enzymes (Phenylalanine ammonia layase (PAL) and chitinase) and salicylic acid (SA). The different enzymes increased 96 h after inoculation with the pathogen and then decreased. The enzymes content was increased in inoculated seedling compared with the un-inoculated ones. The highest SA content was found in Giza 178 rice cultivar.

Keywords: Rice; Blast disease: *Pyricularia grisea*; Biochemical; varieties

INTRODUCTION

The most important and affective disease in rice crop worldwide is blast and the caused by *P. grisea* Sace., (Osman *et al.* 2002. and Scheuermann *et al*, 2012). This disease caused yield losses each year are enough to feed more than 60 million people (Scheuermann *et al*, 2012 and Divya *et al*, 2014). The causal fungus is *Magnaporthe oryzae* as ascomycetes, (anamorph *Pyricularia oryzae*) and *M. grisea* (anamorph *P. grisea*) a new species based on mating types for the fungus experiments (Samalova *et al*, 2014). The symptoms for this disease appear on vegetative parts, other parts during maturing stage of the crop. Identification of sources of resistance is necessary to manage the disease. New genotypes for host resistance are the best way to manage the different disease (Bonman and Nelson, 1992). The development of rice genotypes may provide new methods to control blast disease and increasing the approaches involved in defense to infection with *P. grisea*. The activation of defense response pathways is the target to make plants defend themselves against pathogens (Staskawicz *et al*, 1997). The activities of peroxidase (POX) and polyphenol oxidase (PPO) increased depending on the systemic induced resistance process. Catalyzing lignin formation is responsibility for these enzymes. Biosynthesis process as phytoalexins and phenols is responsible for phenylalanine

ammonia lyase (PAL). β-1,3-glucanase and chitinase enzymes that belonging to pathogenesis-related proteins (PRPs) like PR-3 and PR-2 (van Loon *et al.,* 2006). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are performed by these enzymes and all these enzymes shown linked with plant defense against pathogens in many pathosystem (Kini *et al.,* 2000). Plants can induced some related antioxidant defense systems under biotic and abiotic stresses and that respond to decreasing damage effects of reactive oxygen species (ROS) (Panda, 2007). ROS-scavenging enzymes   may decrease the extent of oxidative damage and the levels of ROS in plant cell (Liang *et al.,* 2003).

The aim of this work was to evaluate some rice genotypes for blast disease and identification some *P. grisea* isolates*.* Also, determination of some biochemical changes in rice seedling after inoculation with the *P. grisea*.

MATERIALS AND METHODS

The research work was established at rice pathology laboratory, greenhouse and farm at Kafr Elsheikh, Sakha, Rice Research and Training Center (RRTC) in two rice growing seasons (2016 and 2017).

Five rice lines from each cross i.e Giza 178 X CT9737-6-1-1-6-3P-M, these two parents resistant to blast disease, the second cross Sakha 101(susceptible) X Sakah 105 (resistant) and the third cross as Sakha 104 (susceptible) X WAB450-I-B-P-106-HB (susceptible). All the parents form Egyptian rice entries except CT9737-6-1-1-6-3P-M from Colombia (Table 1).

Blast sample collection: Rice blast Samples were collected during growing seasons (2015 and 2016) from Kafr El-Sheikh, Gharbia, Sharkia, Dakahlia, Damietta and Beheira governorates.

Table 1. Parentage, origin and grain type of evaluated rice genotypes

Parents	Improved line	Type
Giza 178 (R*) X CT9737-6-1-1-6-3P-M (R)	GZ10499-4-1-1-1 GZ10499-4-1-1-3 GZ10499-4-1-2-1 GZ10499-9-1-1-1 GZ10499-12-1-1-1	IJ
Sakha 101 (S**) X Sakha 105 (R)	GZ10501-2-6-3-2 GZ10501-13-1-1-1 GZ10501-15-2-1-2 GZ10501-15-2-3-2 GZ10501-15-3-1-8	J
Sakha 104 (S) X WAB450-I-B-P-106-HB (S)	GZ10686-2-1-2-1 GZ10686-2-1-2-3 GZ10686-2-1-3-4 GZ10686-11-1-1-3 GZ10686-11-1-1-6	IJ

*R, resistant; **S, susceptible. Indic Japonica (IJ) , Japonica (J)

Isolation of rice blast fungus:

Typical blast lesions on leaves and panicles were isolated according to Shabana *et al* (2013). The isolates were grown and multiplied on banana medium under light florescent for ten days at 28°C for spore production. The isolates were collected at least 25 spores per microscopic at 10x objective (Shabana *et al* (2013).

Pathogenicity test and identification of blast physiological races: Fifteen rice lines, with their six parents as well as, eight international differential varieties (I.D.V) (Atkins *et al,* 1967) were used to evaluate and identify physiological blast races. All tested entries were inoculated with twenty isolates under greenhouse conditions. The tested entries were seeded in plastic trays (30 x 20 x15 cm). Each tray comprised 20 rows, with three replicates (Sehly *et al*, 2008). The trays were fertilized as recommended and then placed in the greenhouse at 28±2 °C. The different seedling at 3 to 4 leaf stage (about 3-4 weeks after sowing) were inoculated with spore suspension at ($5x10^4$ spores/ml) using electrical spray gun. The inoculated seedlings were kept in a moist chamber 95% R.H. and 28±2°C for 24 hr and then put in greenhouse with similar conditions.

Disease assessment: Seven days after inoculation under greenhouse conditions, blast reactions as blast typical lesions were scored as 0-9 scale according to IRRI, 1996.

Biochemical studies:

Enzyme Activity: Rice leaves of different genotypes were collected after pathogen inoculation with the selected virulent race no.1 (IC-3) at various time intervals (48, 96 and 120 hr) and were quickly frozen in liquid nitrogen and stored at -20°C. Three replicates were maintained for each rice genotype.

Peroxidase (POX) activity: POX enzyme activity was determined according to Allam and Hollis (1972) and the methods described by and Srivastava (1987). POX activity was determined as changes in absorbance (optical density/min/0.5g) and activity of POX counted by /molar absorption coefficient *(U* = 26.6 mM^{-1} cm^{-1}). The absorbance using a spectrophotometer was measured at 425 nm.

Ascorbate peroxidase (APX) activity was assayed by the method according to Nakona and Asada (1981) and the absorbance was measured after 30 s of decreased at (290 nm) spectrophotometer. Molar extinction was using by coefficient (U = 2.8 mM^{-1} cm^{-1}) to determine the activity.

Phenylalanine ammonia layase activity (PAL) was determined by the method of Campos *et al.* (2004) and the absorbance was measured at 290 nm. PAL activity was counted by (μ moles of trans-cinnamic acid released / min/ mg proteins) under the specific condition.

Chitinase assay: activity of chitinase was assessed according to Giri et al. (1998). The chitin powder was used as substrate (colloidal chitin) and prepared by the method described according to by Ried and Ogrud (1981). The chitinase activity was measured as O.D. at 540 nm (µg N-acetyl glucosamine released / min / mg of protein).

Salicylic acid aAssay (SA): Samples of rice seedling were taken after 2 days from inoculated and non-inoculated with IC-3 race of *P. grisea* to assay the SA. Rice samples extraction and quantification of SA was conducted in the Food Technology Res. Institute, Agric. Res. Center according to (Goupy *et al*, 1999).

Data Analysis: Data of enzymes determination were subjected to randomized completely design (RCD) Duncan, (1955) .

RESULTS AND DISCUSSION

Isolation of the causal organism of blast disease:

Rice infected samples with blast collected from different rice cultivars and different locations during 2015-2016 seasons are shown in Table (2). Twenty *P. grisea* isolates were successfully isolated and purification was by the single spore technique. These isolates were identified to eleven races using the IDV under greenhouse conditions data in Table (3). Rice blast fungus, *P. grisea* is known to be highly variable (Sehly *et al*. 2008). Many investigators studied the physiological races of the fungus at different rice-growing areas (Arafa, 2012 and Shabana *et al*, 2013).

Pathogenicity test and race identification

Results indicated that the twenty isolates were identified as five groups using the IDV (Table 3). Thirteen isolates were identified as group IC, while four isolates were identified as group ID race. On the other hand, isolates no 3, 7 and 18 were identified as IB, IA and IF group races. These results are agreed with the findings of (Sehly *et al,* 2008; Arafa 2012 and Shabana *et al*, 2013) that showed the distribution of races at different governorates. The virulence of the obtained isolates was determined by inoculating the fifteen rice lines, with their six parents of each individual isolate. The twenty isolates except isolates no. 9, 11 and 14 were virulent to Sakha 101(the commercial rice cv) (Table 4). While, only 13 isolates were virulent on Sakha 104 and no isolates were able to infect Giza178 or Sakha 105 rice cv (Table 4). All lines were resistant for the blast fungus except GZ10686-2-1-2-3 and GZ10686-11-1-1-3 that were susceptible to five isolates, while, GZ10686-2-1-3-4 was susceptible to six isolates. These results will be considered in developing resistant genotypes which is the most important method for control rice blast disease. This approach is friendly for environment and disease mangement (Bonman *et al,* 1992). Moldenhauer *et al.* (1992) evaluated nine parents to rice blast races IB-49 and IC-17. Parents, generally

showed resistance for rice blast fungus races (IC-17 and IB-49), that was monogenic and dominant under the natural conditions. Wang *et al.* (2007) inoculated 141 rice entries with blast race IC-17 to verify the presence of Pi-ta gene, 41 accessions were resistant. Sedeek *et al*. (2015) evaluated 10 promising lines and their parents for rice blast disease. The breeding lines were resistant to rice blast under artificial inoculation in greenhouse and field condition, but Sakha 101were susceptible. Fang et al. (2017) reported that rice varieties were resistant to different races of *M. oryzae* in Australia. The variety SHZ-2 exhibited a resistant reaction to all five races, while, BR-IRGA-409, Ceysvoni, Rikuto Norin 20, NTR587 and Kyeema, were resistant to *M. oryza* at least three races.

Table 2.Source of *Pyricularia grisea* isolates collected in 2015 and 2016 seasons

Isolate no.	Governorate	District	Rice cultivar/line	Year	Race
1	Kafr El-Sheikh	Desouq	Sakha 101	2015	**IC-3**
2	Kafr El-Sheikh	Desouq	Sakha l01	2016	**IC-3**
3	Kafr El-Sheikh	Sakha	Sakha 104	2015	**IB-47**
4	Kafr El-Sheikh	Foaa	Sakha 101	2015	**IC-3**
5	Kafr El-Sheikh	Kafr El-Sheikh	Sakha 101	2016	**IC-11**
6	Kafr El-Sheikh	Desouq	Sakha l04	2016	**IC-9**
7	Kafr El-Sheikh	Sakha	Sakha 101	2016	**IA-67**
8	Dakahlia	Talkha	Sakha 101	2015	**ID-11**
9	Dakahlia	Kafr Saad	Sakha 104	2015	**IC-3**
10	Dakahlia	Dekerns	Sakha 101	2016	**ID-3**
11	Dakahlia	Dekerns	Sakha l04	2016	**IC-11**
12	Dakahlia	Talkha	Sakha 101	2016	**IC-11**
13	Beheira	Elebrahimyia	Sakha 104	2015	**ID-15**
14	Beheira	Mahmoudia	Sakha 101	2015	**ID-11**
15	Beheira	Kafr El-Dawar	Sakha 101	2015	**IC-1**
16	Beheira	Itai-El-Barood	BL – 1	2016	**IC-11**
17	Beheira	Itai-El-Barood	Giza171	2016	**IC-3**
18	Beheira	Kafr El-Dawar	Reiho	2016	**IF-1**
19	Damietta	Zarka	Sakha 101	2016	**IC-3**
20	Sharkia	Kafr Saker	Sakha 101	2016	**IC-7**

Table 3. Rice blast reactions on the international differential varieties (IDVs) tested under greenhouse condtions

IDV	Group races	Isolate number/ Reaction																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Raminad str.3	IA	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Zenith	IB	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
NP-125	IC	S	S	R	S	S	S	S	R	S	R	S	S	R	R	S	S	S	R	S	S
Usen	ID	HS	S	S	S	HS	S	S	HS	HS	S	HS	S	HS	S	S	HS	S	R	S	S
Dular	IE	S	S	R	S	R	R	S	R	S	S	R	R	R	R	S	R	S	R	S	S
Kanto 51	IF	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	R
CI 8970 s	IG	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	S	R	R
Caloro	IH	S	S	S	HS	HS	HS	S	HS	S	S	HS	HS	HS	S	HS	S	HS	S	HS	HS
Race		IC-3	IC-3	IB-47	IC-3	IC-11	IC-9	IA-67	ID-11	IC-3	ID-3	IC-11	IC-11	ID-15	ID-11	IC-1	IC-11	IC-3	IF-1	IC-3	IC-7

* Reactions: R = resistant (1-2), MR = moderately resistant (3), S = susceptible (4-6), HS = highly susceptible (7-9).

Table 4. Reaction of 21 rice entries inoculated with 20 *Pyricularia grisea* isolates under greenhouse conditions

No.	Cultivar/entry	IC-	IC-	IB-	IC-	IC-	IC-	IA-	ID-	IC-	ID-	IC-	IC-	ID-	ID-	IC-	IC-	IC-	IF-	IC-	IC-
1	**Giza 178**	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2	**CT9739-6-1-1-6-**	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
3	GZ10499-4-1-1-1	MR	MR	R	R	MR	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R
4	GZ10499-4-1-1-3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
5	GZ10499-4-1-2-1	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R
6	GZ10499-9-1-1-1	R	R	R	R	MR	R	MR	R	R	MR	R	MR	R	R	R	R	R	R	R	R
7	GZ10499-12-1-1-1	MR	MR	MR	R	MR	MR	MR	R	MR	R	MR	MR	R	R	MR	R	R	MR	R	MR
8	**Sakha 101**	S	S	S	S	S	S	HS	HS	R	HS	R	HS	S	R	HS	S	HS	S	HS	HS
9	**Sakha 105**	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R
10	GZ10501-2-6-3-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
11	GZ10501-13-1-1-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
12	GZ10501-15-2-1-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
13	GZ10501-15-3-3-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
14	GZ10501-15-3-1-8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
15	**Sakha 104**	HS	HS	HS	S	HS	HS	R	R	HS	R	HS	S	S	R	R	HS	S	HS	R	R
16	**WAB450-I-B-P-**	S	S	R	R	S	R	S	R	R	R	HS	R	R	R	R	S	R	R	R	R
17	GZ10686-2-1-2-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
18	GZ10686-2-1-2-3	S	R	S	R	R	S	R	R	S	R	S	R	MR	R	R	MR	R	R	R	R
19	GZ10686-2-1-3-4	S	R	S	R	R	S	R	R	S	R	S	R	S	R	R	MR	R	R	R	R
20	GZ10686-11-1-1-3	S	R	S	R	R	S	R	R	S	R	S	R	MR	R	R	MR	R	R	R	R
21	GZ10686-11-1-1-6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

* Reactions: R = resistant (1-2), MR = moderately resistant (3), S = susceptible (4-6), HS = highly susceptible (7-9)

Activity of antioxidative enzymes:

Peroxidase activity (POX) ranged from 460.2 to 1510.0µ moles H_2O_2 oxidized in the inoculated seedling (Fig. 1). While, in un-inoculated leaf samples, the POX activity ranged from 101.0 to 516.0 µ moles H_2O_2 oxidized. The maximum increase in POX activity was recorded at 96 hr after inoculation and then decreased. The highest activity for POX significantly induced in blast resistant genotype) in GZ 10686-2-1-2-1, GZ 1068-11-1-1-6, GZ 10501-13-1-1-1 and GZ10501-15-3-3-2. The least level of induction was recorded in Sakha 104 and 101 (231.4 and 250.0 µ moles, respectively), a susceptible rice cultivars. POX enzyme in healthy plants may be due to their presence in healthy plant tissues as constitutive enzymes. The second messengers in resistance mechanisms is reactive oxygen species (ROS), it is considered indicator to interfere with other important signaling molecules and the defense-related genes (Chen *et al,* 2014). Enzymatic antioxidants such as peroxidase (POX) can control production of ROS during plant -pathogen interaction (Barna *et al,* 2012). Activation of POX enzyme in the various substrates using hydrogen peroxide can catalyzes oxido-reduction and elimination of ROS. POX may acts as either H_2O_2 scavenger or generator depending on physiological condition (Almagro *et al,* 2009). POX activity induced may be elimination the generation of ROS and control it (Rahman and Wenner, 2014) or appear toxicity toward invading pathogen (Torres 2006).

APX activity profile ranged from 105.6 to 254.8 µ moles ascorbate oxidized (ASO) in the un-inoculafrom between 380.0 to 1663.2 µ moles (ASO) (Fig. 2). APX activity recorded higher levels after 96 hr of inoculation with the blast pathogen. There were no significant difference among Giza 105, Giza 178 and CT9739-6-1-1-6-3p-M (1663.2, 1523.0 and 1492.4 µ moles (ASO), respectively). These genotypes proved to be the highest activate for APX and resistance to blast disease. Sakha 101 and 104 recorded lower levels of APX after 96h. APX activity significantly increased in blast resistant genotypes after 48 and 96h and then decreased. APX activity is the one of the most important antioxidant enzymes, practically all sub-cellular compartments. Agrawal (2003) suggested that cytosolic APX genes are up regulated upon wounding and plays as a protective role against pathogens in rice. These enzymes may arrest the ROS production with higher levels in resistant rice genotypes compared with susceptible ones. These results suggest the critical role of early O_2- and H_2O_2 accumulation; also POX and APX may help to lignification as defense mechanism involved in basal resistance in our pathosystem. Using POX for H_2O_2 to has oxidized for some phenolics constituents to lignin (Sharma *et al,* 2012; Nikraftar *et al,* 2013).
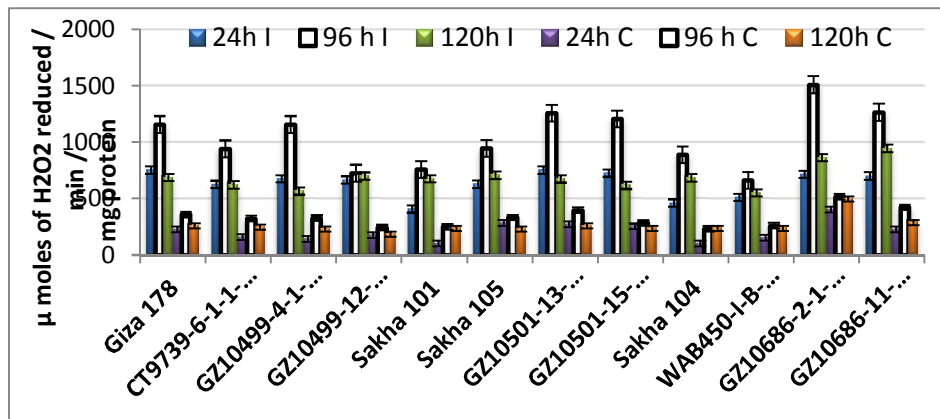
Fig. 1. Levels of peroxidase activity in leaves of rice genotypes 48, 96 and 120 hr after artificial inoculation with *P. oryzae.* The bar represent standard error (SE ±) of mean (n = 3) at Duncan test (P≤0.05= 153.7) probability.
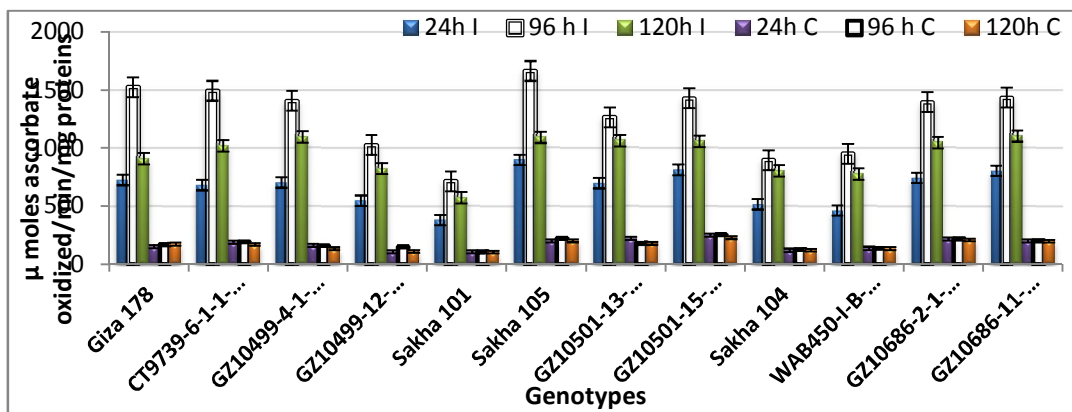

Fig. 2. Levels of ascorbate peroxidase activity in leaves of rice genotypes 48, 96 and 120 hr after artificial inoculation with *P. oryzae*. The bar represent standard error (SE ±) of mean (n = 3) at Duncan test (P≤0.05= 212.8) probability.

Profile activity of defense-related enzymes

The PAL activity profile of the 21 rice genotypes ranged from 0.6 to 1.83 μ moles cinnamic acid in un-inoculated while it ranged from 1.07 and 5.20 μ moles cinnamic acid in the inoculated seedling (Fig. 3). The PAL activity was significantly higher in GZ10501-13-1-1-1 after 96hr and then decreased after 120hr (5.20 and 3.527 μ moles cinnamic acid). The PAL increased in inoculated resistant genotypes (GZ10686-11-1-1-6 followed by Sakha 105 and GZ 10501-15-3-3-2), and the low level was recorded in Sakha 101 blast susceptible (Fig. 3). Also, PAL activity constitutively recorded higher levels in three groups of genotypes. New proteins are formed in interactions between plant-pathogens direct or indirect effect in plant resistance to pathogen called as pathogenesis- related (PR) proteins (Jones & Dangl, 2006). Some PR protein like *β*-glucosidase, PAL and chitinase were found in plant resistance against pathogens

fungal (Kini *et al*, 2000). PAL catalysis is responsible for biosynthesis of the phenyl propanoid pathway and involved in the synthesis of twice phytoalexin and lignin. These phytoalexin and lignin can prevent penetration by the pathogen for cell wall (Dixon, 2001). Zhang and Yu (1987) found that increase the level of PAL in rice cultivars (six) was different in level of resistance to *M. oryzae*. Hsieh *et al*. (2010) reported that PAL activity in resistant cultivars was higher than in susceptible ones which may be due to difficult of site infection and preventing pathogen to entry the host plant.

The activity of chitinase ranged from 4.4 and 33.7 µgNAG in un- inoculated 21 rice genotypes. The activity of chitinase increased after inoculation with *P. oryzae* from 8.3 to 55.0 µg NAG (Fig. 4). The activity of chitinase was highert in inoculated GZ10-686-11-1-1-6 and Giza 178 as (55.0 and 53.8 µg NAG). While, WAB450-I-B-P-106-HP recorded lower activity after inoculation compared to other rice genotypes. Chitinase activity was higher in infected and wounded plants compared with healthy ones (Boller, 1988). Plants can use some receptors recognized to defend themselves from microbial pathogens. These receptors recognize pathogen-associated molecular patterns and activate signaling pathways that lead to immunity. In rice the chitin elicitor binding protein recognizes, oligosaccharides and released from the cell walls of fungal pathogens. Here, we show this first line of plant defense against rice blast fungus *M. oryzae* by secreting an effector protein, secreted LysM Protein1 (Slp1), during invasion of new rice cells. Accumulation of the Slp1 at the interface between the fungal cells well and the rice plasma membrane can bind to chitin, and is able to suppress chitin-induced plant immune responses, including generation of reactive oxygen species and plant defense gene expression (Thomas *et al,* 2012).
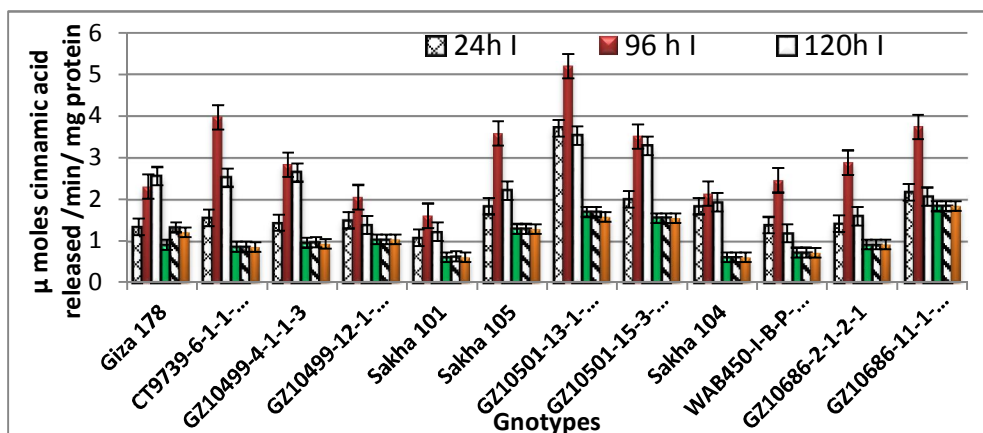

Fig. 3. Levels of PAL in leaves of rice genotypes 48, 96 and 120 hr after artificial inoculation with *P. oryzae*. The bar indicates standard error (SE ±) of mean (n = 3) at Duncan test (P≤0.05= 0.255) probability.
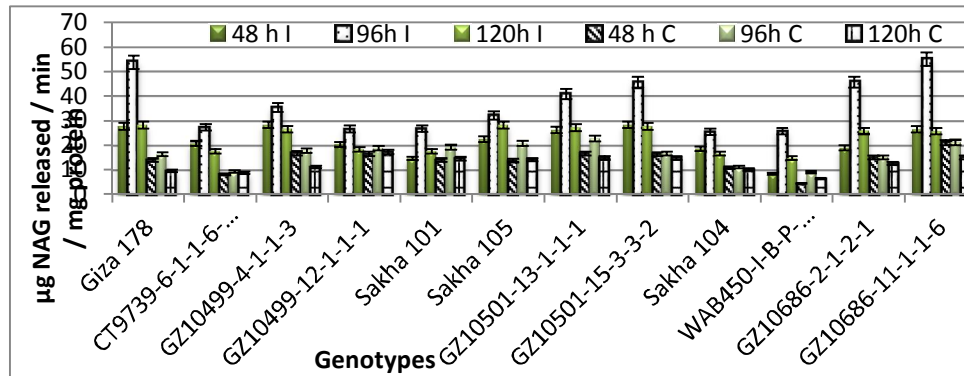
Fig. 4. Levels of chitinase activity in leaves of rice genotypes 48, 96 and 120 hr after artificial inoculation with *P. oryzae.* The bar indicates standard error (SE ±) of mean (n = 3) at Duncan test (P≤0.05= 7.23 probability).

Shimizu *et al*. (2010) reported that Secreted LysM Protein1 considered a novel effector secreted by *M. oryzae.* In rice LysM Protein1 required for chitin cooperation to induced kinase Os-CERK1.

Activity of defense-related Salicylic Acid (SA):

The SA content of inoculated rice genotypes after 48hr ranged between 13.87 and 40.97 µg/mg. SA content was higher in inoculated seedlings compared with un-inoculated (Fig.5). Giza 178 (Resistant cultivar) proved to have the highest content of SA in inoculated and un-inoculated (40.97 and 35.28µg/mg) with the pathogen. There was no significant difference between Giza 178 and GZ10501-15-2-1-2 (R) in SA content. The low level of induction was recorded in inoculated Sakha 101 and 104 (13.87 and 14.9 µg/ mg, respectively) and un-inoculated (7.15 and 8.83 µg/ mg, respectively), blast susceptible rice cultivars. The most important role for SA is signaling the induction of defense responses for various plants after pathogen infection, and these responses include; induced local and systemic resistance disease, potentiation of host cell death, and the containment of pathogen spread. The effects of SA mediate may appear on increased defense gene expression, alterations in the activity or synthesis of certain enzymes, potentiation of several defense responses, and/or the generation of free radicals (Dempsey *et al.,* 1999).
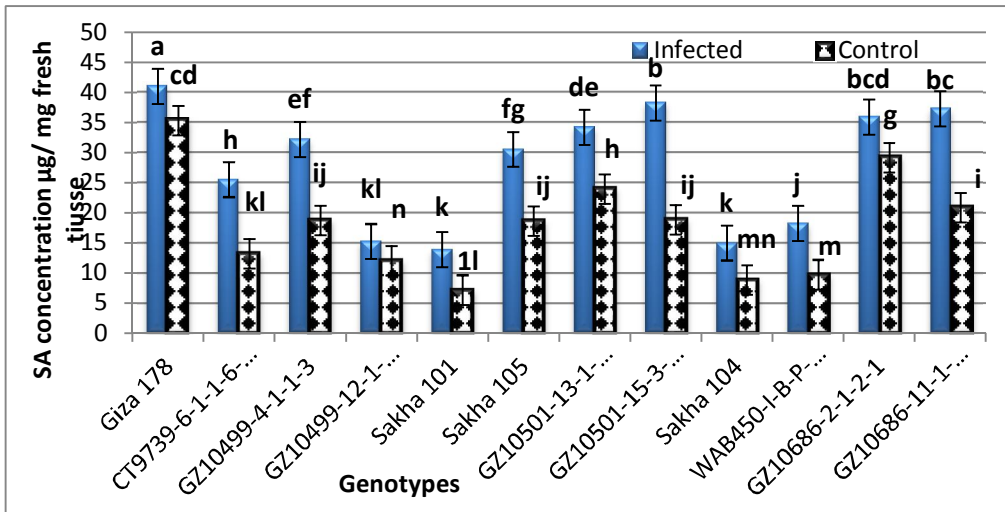
Fig. 5. Salicylic acid concentration in leaves of rice genotypes and after artificial inoculation with *P. oryzae*. The bar indicates standard error (SE ±) of mean (n = 3) in a column, means followed by a common letter are not significantly different at Duncan test with P≤0.05 probability.

Salicylic acid (SA; 2-hydroxybenzoic acid) is one of many phenolic compounds that are synthesized by plants. Phenolic compounds may be involved in many important processes, including lignin and pigment biosynthesis, allelopathy, and the regulation of responses to abiotic and biotic stress (Métraux, 1993).

The activities of defense-related and antioxidant enzymes in resistant genotypes increased in association with *P. grisea* infection. This suggesting that resistant rice genotype has strong defense systems. In susceptible rice genotypes, the activities of defense-related and antioxidant enzymes were lower compared with resistant genotypes. These results could be effectively exploited to screen the rice genotypes for blast resistance.

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مقاومة طرز وراثية من الأرز لفطر اللفحه و
التغيرات البيوكيميائية المصاحبه

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يعتبر مرض لفحة الارز من اهم امراض الارز في مصر والعالم. الفطر المسبب لمــرض اللفحه *Pyricularia grisea* لــه القدره علي انتاج العديد من السلالات الفسيولوجيه. من اهــم الطــرق الفعاله في مقاومة المرض هي انتاج اصناف ارز تحمل صفات المقاومه المستدامه. تركــزت هــذه الدراسه علي تعريف 20 عزله من الفطر الممرض وتقييم 21 نمط وراثي للعدوي بالفطر المســبب. و ايضا دراسة التغيرات الكيميائية الحيوية بعد العدوي بالفطر الممرض. قسم تعريف السلالات الــي 5 مجموعات، وفكانت السلاله الشائعه (IC)، يليها ID وسلاله واحده فقط لكلا مــن IA، IB و IF. لوحظ في تقييم الطرز الوراثيه في الارز ان المجموعه الاولي والثانيه مقاومه للاصابه بالمرض، اما المجموعه الثالثه ترواحت المقاومه 70 الي 75 %. التغيرات الكيميائية الحيوية تم دراستها بواسطة تقدير انزيمات مضدات الاكسده (انزيمي البيروكسيديز و اسكوربك بيروكسيديز)، انزيمات مرتبطــه بالدفاع (انزيمي الفينيل الالانين امونياليز و الكيتين) وحمض السلسيلك. زادت الانزيمات المقدره بعد 96 من العدوي بالفطر المسبب وبعد ذلك انخفضت. محتوي بادرات الارز الملقحه بالفطر المســبب من الانزيمات اعلي من الغير ملقحه. وجد ان اعلي محتوي لحمص السلسيلك في صنف الارز جيزه 178.